

Please replace the paragraph starting at page 21 line 1 with the following rewritten paragraph:

Also provided herein are purified Hu-Asp polypeptides, both recombinant and non-recombinant. Most importantly, methods to produce Hu-Asp2 polypeptides in active form are provided. These include production of Hu-Asp2 polypeptides and variants thereof in bacterial cells, insect cells, and mammalian cells, also in forms that allow secretion of the Hu-Asp2 polypeptide from bacterial, insect or mammalian cells into the culture medium, also methods to produce variants of Hu-Asp2 polypeptide incorporating amino acid tags that facilitate subsequent purification. In a preferred embodiment of the invention the Hu-Asp2 polypeptide is converted to a proteolytically active form either in transformed cells or after purification and cleavage by a second protease in a cell-free system, such active forms of the Hu-Asp2 polypeptide beginning with the N-terminal sequence TQHGIR (SEQ ID NO: 56) or ETDEEP (SEQ ID NO: 57). Variants and derivatives, including fragments, of Hu-Asp proteins having the native amino acid sequences given in SEQ ID Nos: 2, 4, and 6 that retain any of the biological activities of Hu-Asp are also within the scope of the present invention. Of course, one of ordinary skill in the art will readily be able to determine whether a variant, derivative, or fragment of a Hu-Asp protein displays Hu-Asp activity by subjecting the variant, derivative, or fragment to a standard aspartyl protease assay. Fragments of Hu-Asp within the scope of this invention include those that contain the active site domain containing the amino acid sequence DTG, fragments that contain the active site domain amino acid sequence DSG, fragments containing both the DTG and DSG active site sequences, fragments in which the spacing of the DTG and DSG active site sequences has been lengthened, fragments in which the spacing has been shortened. Also within the scope of the invention are fragments of Hu-Asp in which the transmembrane domain has been removed to allow production of Hu-Asp2 in a soluble form. In another embodiment of the invention, the two halves of Hu-Asp2, each containing a single active site DTG or DSG sequence can be produced independently as recombinant polypeptides, then combined in solution where they reconstitute an active protease.

Please replace the paragraph starting at page 27 line 7 with the following rewritten paragraph:

A3 The polypeptides of the present invention may also be used to raise polyclonal and monoclonal antibodies, which are useful in diagnostic assays for detecting Hu-Asp polypeptide expression. Such antibodies may be prepared by conventional techniques. See, for example, *Antibodies: A Laboratory Manual*, Harlow and Land (eds.), Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y., (1988); *Monoclonal Antibodies, Hybridomas: A New Dimension in Biological Analyses*, Kennet et al. (eds.), Plenum Press, New York (1980). Synthetic peptides comprising portions of Hu-Asp containing 5 to 20 amino acids may also be used for the production of polyclonal or monoclonal antibodies after linkage to a suitable carrier protein including but not limited to keyhole limpet hemacyanin (KLH), chicken ovalbumin, or bovine serum albumin using various cross-linking reagents including carbodimides, glutaraldehyde, or if the peptide contains a cysteine, N-methylmaleimide. A preferred peptide for immunization when conjugated to KLH contains the C-terminus of Hu\_Asp1 or Hu-Asp2 comprising QRRPRDPEVVNDESSLVRHRWK (SEQ ID NO: 50) or LRQQHDDFADDISLLK (SEQ ID NO: 51), respectively.

Please replace the paragraph starting at page 28 line 1 with the following rewritten paragraph:

A4  
Cm't In another embodiment, the invention relates to a method of assaying Hu-Asp function, specifically Hu-Asp2 function which involves incubating in solution the Hu-Asp polypeptide with a suitable substrate including but not limited to a synthetic peptide containing the  $\beta$ -secretase cleavage site of APP, preferably one containing the mutation found in a Swedish kindred with inherited AD in which KM is changed to NL, such peptide comprising the sequence SEVNLDAEFR (SEQ ID NO: 54) in an acidic buffering solution, preferably an acidic buffering solution of pH5.5 (see Example 12) using cleavage of the peptide monitored by high performance liquid chromatography as a measure of Hu-Asp proteolytic activity. Preferred assays for proteolytic activity utilize internally quenched peptide assay substrates. Such suitable substrates include peptides which have attached a paired fluorephore and quencher including but not limited to coumarin and dinitrophenol, respectively, such that cleavage of the peptide by the Hu-Asp results in increased fluorescence due to physical

A4  
cncld.

separation of the flurophore and quencher. Preferred colorimetric assays of Hu-Asp proteolytic activity utilize other suitable substrates that include the P2 and P1 amino acids comprising the recognition site for cleavage linked to o-nitrophenol through an amide linkage, such that cleavage by the Hu-Asp results in an increase in optical density after altering the assay buffer to alkaline pH. *m*

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Please ~~replace~~ the paragraph starting at page 57 line 8 with the following rewritten paragraph:

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A5

--Sequence analysis of the purified Hu-Asp2 $\Delta$ TM(His)<sub>6</sub> protein revealed that the signal peptide had been cleaved [TQHGIRLPLR] (SEQ ID NO: 52).--

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ALC  
"TQHGIRLPLR"

Please ~~replace~~ the paragraph starting at page 59 line 15 with the following rewritten paragraph:

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**Example 12. Assay of Hu-Asp2  $\beta$ -secretase activity using peptide substrates**

*$\beta$ -secretase assay*— $\beta$ -secretase activity was measured by quantifying the hydrolysis of a synthetic peptide containing the APP Swedish mutation by RP-HPLC with UV detection. Each reaction contained 50 mM Na-MES (pH 5.5), 1%  $\beta$ -octylglucoside, peptide substrate (SEVNLDAEFR, 70  $\mu$ M; SEQ ID NO: 54): and enzyme (1-5  $\mu$ g protein). Reactions were incubated at 37 °C for various times and the reaction products were resolved by RP-HPLC using a linear gradient from 0-70 B over 30 minutes (A=0.1% TFA in water, B=).1%TFA/10%water/90%AcCN). The elution profile was monitored by absorbance at 214 nm. In preliminary experiments, the two product peaks which eluted before the intact peptide substrate, were confirmed to have the sequence DAEFR and SEVNL using both Edman sequencing and MADLI-TOF mass spectrometry. Percent hydrolysis of the peptide substrate was calculated by comparing the integrated peak areas for the two product peptides and the starting material derived from the absorbance at 214 nm. The specificity of the protease cleavage reaction was determined by performing the  $\beta$ -secretase assay in the presence of a cocktail of protease inhibitors (8  $\mu$ M pepstatin A, 10  $\mu$ M leupeptin, 10  $\mu$ M E64, and 5 mM EDTA). *m*

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